ROSS PRODUCTS DIVISION • ABBOTT LABORATORIES

625 CLEVELAND AVENUE • COLUMBUS, OHIO 43215-1724 • (614) 624-7677

OVERNIGHT MAIL

October 21, 2005

Ms. Felicia Billingslea (HFS-820)
Director, Food Labeling and Standards Staff, Room 4D-045
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

RE: Notification Submitted Pursuant to Section 403(w)(7) of the Food Allergen Labeling and Consumer Protection Act

The enclosed notification pertains to the extensively hydrolyzed casein ingredient used in Similac® Alimentum® Advance® and is submitted pursuant to the procedures established by the Food Allergen Labeling and Consumer Protection Act under 21 U.S.C. 343(w)(7).

An original with three copies are enclosed in light blue binders for FDA review. In addition, 4 redacted copies have been enclosed to assist in satisfying public requests for Ross' notification. The redacted versions are marked [REDACTED] in the document footer and are enclosed in gray binders. Redacted information is highlighted in the original (yellow highlights) and copies (gray highlights) intended for FDA review.

Thank you for your attention.

Sincerely,

Pamela Anderson, PhD, RD
Director, Regulatory Affairs
Ross Products Division
Abbott Laboratories
625 Cleveland Avenue
Columbus, Ohio 43215-1724

2005FL-0434

FLN 1

NOTIFICATION SUBMITTED PURSUANT TO SECTION 403(w)(7) OF THE FOOD ALLERGEN LABELING AND CONSUMER PROTECTION ACT

Extensively Hydrolyzed Casein In Similac® Alimentum® Advance®

Submitted By:
Ross Products Division
Abbott Laboratories
625 Cleveland Avenue
Columbus, OH 43215

October 21, 2005

NOTIFICATION SUBMITTED PURSUANT TO SECTION 403(w)(7) OF THE FOOD ALLERGEN LABELING AND CONSUMER PROTECTION ACT

Extensively Hydrolyzed Casein In Similac® Alimentum® Advance®

Table of Contents

REDACTED

NOTIFICATION SUBMITTED PURSUANT TO SECTION 403(w)(7) OF THE FOOD ALLERGEN LABELING AND CONSUMER PROTECTION ACT

The Ross Products Division of Abbott Laboratories submits this notification pursuant to Section 403(w)(7) of the Federal Food, Drug, and Cosmetic Act (FFDCA) as amended by the Food Allergen Labeling and Consumer Protection Act (FALCPA). This notification sets forth the scientific evidence and other data establishing that the extensively hydrolyzed casein found in Similac® Alimentum® Advance® Protein Hydrolysate Formula with Iron (Alimentum) infant formula is a "hypoallergenic protein" rather than an "allergenic protein."

As will be discussed in more detail below, FALCPA establishes a notification process for obtaining an exemption from the definition of "major allergen" in those instances when there are data demonstrating that an ingredient derived from a major allergen does not contain "allergenic protein." The data in this notification establish that Alimentum does not contain "allergenic protein."

The American Academy of Pediatrics (AAP) has established the clinical performance criteria for hypoallergenic infant formulas. The criteria include conducting clinical studies demonstrating that the infant formula does not cause an allergic response in most infants with a food allergy. Ross conducted such a clinical study and the Food and Drug Administration (FDA), as part of its review of an infant formula premarket notification, has concurred there are sufficient data to classify Alimentum as a hypoallergenic infant formula under the AAP-recommended standards. Because Alimentum has been demonstrated to be a hypoallergenic infant formula, the protein in Alimentum should not be considered an "allergenic protein" that is subject to the definition of "major food allergen."

We first discuss our basis for concluding that it is appropriate for FDA to review this request under the FALCPA notification provisions and then provide a summary of the scientific evidence supporting our position that the extensively hydrolyzed casein in Alimentum is a hypoallergenic protein.

I. LEGAL BASIS FOR FILING A FALCPA NOTIFICATION

A. Statutory Background

FALCPA establishes labeling requirements for major allergens. FALCPA amended the FFDCA and deems a food misbranded "if it is, or it contains an ingredient that bears or contains, a major food allergen" unless the food is labeled in accordance

with one of two labeling options.1 A "major food allergen" is defined in paragraph (1) of subsection 201(qq) of the FFDCA as milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans, collectively known as the "Big 8 Allergens." A major food allergen also includes "a food ingredient that contains protein derived from a food specified in paragraph (1)," with the exception of highly refined oils and food ingredients that are exempt under the petition and notification procedures found in the statute.2

FALCPA establishes the requirements for determining whether an exemption can be achieved through a notification or petition process.3 A notification is appropriate when there is "(i) scientific evidence (including the analytical method used) that demonstrates that the food ingredient (as derived by the method specified in the notification, where applicable) does not contain allergenic protein; or (ii) a determination by [FDA] that the ingredient does not cause an allergic response that poses a risk to human health under a premarket approval or notification program under section 409."4 In instances when an ingredient does not qualify for the notification process, a petition can be filed requesting FDA to exempt a food ingredient from the labeling requirements by providing "scientific evidence...that demonstrates that such food ingredient...does not cause an allergic response that poses a risk to human health."5

B. Alimentum Does Not Contain Allergenic Protein

The notification process is appropriate when there is scientific evidence establishing that the food ingredient does not contain allergenic protein. While FALCPA does not define "allergenic protein," the definition reasonably should not encompass the extensively hydrolyzed casein in Alimentum, which has been demonstrated to be hypoallergenic. The AAP has established criteria for determining whether an infant formula could be positioned as "hypoallergenic." The AAP concludes:

> To be labeled as hypoallergenic, these formulas, after appropriate preclinical testing, must demonstrate in clinical studies that they do not provoke reactions in 90% of infants or children with confirmed...allergy with 95% confidence when

---

1 FFDCA § 403(w)(1). One option involves placing the word "contains," followed by the name of the food source from which the major allergen is derived, either immediately after or adjacent to the ingredient statement. The other option involves including the name of the food source from which the major allergen is derived in a parenthetical following the listing of the ingredient in the ingredient statement, except that such parenthetical listing is not necessary when the name of the food source appears as part of the listing of another major allergen in the food product. See id.

2 FFDCA § 201(qq)(2).

3 See FFDCA § 403(w)(6) and (7).

4 FFDCA § 403(w)(7)(A) (emphasis added).

5 FFDCA § 403(w)(6)(C).

given in prospective randomized, double-blind, placebo-controlled trials.6

Ross conducted a double-blind placebo controlled food challenge (DBPCFC) trial that corresponds to the AAP criteria and demonstrates that milk allergic infants can consume Alimentum. Ross then submitted to FDA, under Section 412 of the FFDCA, an infant formula premarket notification for this "exempt infant formula." The premarket notification contained the preclinical and clinical data outlined in the AAP criteria for the labeling and use of a hypoallergenic infant formula. FDA completed a favorable review of the premarket notification in 1989 and subsequently their favorable review of hypoallergenic/cow milk allergy claims in 1990.

The applicable issue here is whether Alimentum contains "allergenic protein." To the extent that it "does not contain an allergenic protein," it is appropriate to obtain an exemption from the major allergen definition under the FALCPA notification process. Hypoallergenic infant formulas are developed and specifically marketed for use by infants with food allergies. These infant formulas are considered "hypoallergenic" because the vast majority of infants with food allergies will be able to ingest the product without suffering an adverse reaction. Unlike hypoallergenic proteins, an allergenic protein will consistently induce an adverse reaction when consumed by an individual that is sensitized to that allergen. Given the use of hypoallergenic infant formulas by infants with milk allergies, "hypoallergenic proteins" should not be treated as "allergenic proteins" under FALCPA.

We recognize there is a very small number of exquisitely sensitive milk-allergic infants who will be unable to consume a hypoallergenic infant formula. This factor, however, does not preclude a finding that the product does not contain allergenic protein. FALCPA does not require a finding that an ingredient is incapable of inducing an allergic response. The notification and petition provisions both recognize that FDA should exempt an ingredient from the definition of "major allergen" when there are data demonstrating that the food ingredient "does not cause an allergic response that poses a risk to human health."7

Given the recognition by the scientific and medical communities that hypoallergenic infant formulas can be marketed and labeled for use by infants with food allergies, there are sufficient data demonstrating that a hypoallergenic infant formula does not cause an allergic response that poses a risk to human health. Such a conclusion is further supported by the manner in which hypoallergenic infant formulas are administered. Hypoallergenic infant formulas generally are first recommended after the pediatrician has diagnosed the infant's food allergy. The pediatrician will recommend the use of the hypoallergenic infant formula and will monitor the infant closely for an

---

6 American Academy of Pediatrics, Committee on Nutrition, *Hypoallergenic Infant Formulas*, 106 Pediatrics : 346, 348 (Aug. 2000).

7 FFDCA s 403(w)(6)(C), 403(w)(7)(A)(ii).

allergic reaction to the infant formula. In those rare instances when an exquisitely sensitive infant is unable to consume Alimentum, the pediatrician will recommend one of the amino-acid based formulas.

The legislative history provides further support for our position that a notification is appropriate for reviewing hypoallergenic infant formulas. The Senate Committee reporting the bill out of Committee to the full Senate expressly stated that it "encourages FDA to adopt a reasonable standard for determining whether a food ingredient 'does not contain an allergenic protein'...for example, ingredients containing allergenic protein below [a future] established threshold would be eligible for the notification procedure."8 Furthermore, the Senate Committee specifically directed FDA to provide "guidance to industry on the information that would be useful for making a determination that foods that contain protein derived from one of the eight food allergens do not cause an allergic response that poses a risk to human health" and to create "a process...that minimizes the burden on the food manufacturer."9 Given the statutory construct of FALCPA, it would be "reasonable" for FDA to conclude that an infant formula that meets the rigorous requirements established for hypoallergenic infant formulas "does not contain allergenic protein" and can be reviewed under the notification provisions.

C. Alimentum Has Been Reviewed Under a Premarket Notification Program

FALCPA also recognizes that a notification is appropriate in those instances when FDA has concluded that an ingredient "does not cause an allergic response that poses a risk to human health under a premarket approval or notification program under section 409."10 While the extensively hydrolyzed casein in Alimentum has not been reviewed under the food additive provisions of section 409, the hypoallergenic infant formula has been subjected to the rigorous premarket notification process by FDA under section 412.

The agency will consider the requirements of section 409 as part of its review of the premarket notifications submitted for infant formulas. Ross submitted a premarket notification for Alimentum under section 412 and provided the agency with the data and information supporting the marketing of Alimentum as a hypoallergenic infant formula. As part of that premarket notification, Ross submitted data and information supporting the generally recognized as safe (GRAS) status of the extensively hydrolyzed casein in Alimentum. FDA would not have allowed Alimentum to be characterized as "hypoallergenic" unless the agency concluded that the extensively hydrolyzed casein in the formula met the rigorous criteria for hypoallergenic infant formulas. Moreover, because the product is positioned for use by milk allergic infants, FDA reasonably concluded as part of its review under Section 412 that Alimentum would

---

8 S. Rep. No. 108-226 at p.7 (2004).
9 *Id.*
10 FFDCA s 403(w)(7)(A)(ii).

not cause an allergic response that poses a risk to human health. It, therefore, would be reasonable for the agency to take the position that the FALCPA notification requirements of section 403(w)(7)(ii) have been satisfied because there has been finding under Section 409 (through the Section 412 premarket notification process), that the extensively hydrolyzed casein in Alimentum does not cause an allergic response that poses a risk to human health.

D. The Notification Can Focus on the Hypoallergenicity of the Infant Formula

We recognize that the FALCPA notification and petition procedures focus on food ingredients derived from major allergens and not the allergenicity of the finished food products containing such food ingredients. We believe it is appropriate for FDA to consider, as part of the notification and petition programs, data evaluating the allergenicity of the ingredient while in the finished food matrix. Such a consideration is particularly relevant in this instance when the finished food is an infant formula that can serve as the sole source of nutrition for an infant. The most important issue is whether the ingredient derived from the allergen, when eaten by the allergic consumer, would trigger an allergic reaction that would pose a serious risk to health.

The data summarized in Section II establish that Alimentum has undergone the clinical testing demonstrating that it is hypoallergenic and appropriate for consumption by milk allergic infants. The data, therefore, support a finding that the extensively hydrolyzed casein in Alimentum does not contain allergenic protein.

E. An Exemption from the Allergen Labeling Requirements for Hypoallergenic Infant Formulas is Warranted

FDA also would be facilitating the objectives of FALCPA by accepting notifications for hypoallergenic infant formulas. Congress enacted FALCPA to aid consumers and parents of children with food allergies in the identification of foods that contain ingredients derived from major food allergens from which allergic individuals would likely have a serious reaction. The primary legislative goal is to protect human health and provide food allergic consumers with understandable and accurate information about the content of food products so they can choose from a variety of foods that do not pose a serious risk of allergic reaction.

FDA would be sending conflicting signals to parents of milk allergic infants if it subjects Alimentum, a hypoallergenic infant formula, to the allergen labeling requirements. These parents are advised by their physicians to give their child a hypoallergenic formula and to avoid those foods that contain milk ingredients. FDA would create the inaccurate impression that the extensively hydrolyzed casein in Alimentum has the same potential to cause allergic reactions as regular formulas, if it subjects both formulas to the same allergen labeling requirements. FDA, therefore,

would be satisfying the intent of FALCPA by granting an exemption from the allergen labeling requirements for the extensively hydrolyzed casein in Alimentum.

II. SCIENTIFIC SUPPORT FOR THE ESTABLISHMENT OF A THRESHOLD

As described above, it is appropriate to submit a notification to exempt the extensively hydrolyzed casein in Alimentum from the allergen labeling requirements of FALCPA because scientific evidence demonstrates that it no longer has significant allergenic potential and appropriately can be characterized as "hypoallergenic." This determination is based on preclinical and clinical testing of Alimentum in highly atopic infants with cow's milk protein allergy (CMPA). Ross developed these preclinical and clinical data following the protocols established by the AAP and acknowledged by FDA as appropriate for labeling infant formula as "hypoallergenic." The rigor and reliability of the AAP hypoallergenic testing protocol also have been acknowledged by FDA's internal, interdisciplinary Threshold Working Group.

FALCPA requires the notification to contain the scientific evidence, including the analytical method, demonstrating that the food ingredient produced by the method described in the notification does not contain allergenic protein. This Notification satisfies these requirements and contains the following sections: (1) a description of Alimentum, (2) the method used to manufacture the extensively hydrolyzed casein in Alimentum, (3) the Alimentum manufacturing process and the controls in place to ensure that it results in the production of a hypoallergenic formula (4) scientific data demonstrating that the product does not contain allergenic protein, and (5) the analytical method used to demonstrate that the product does not contain allergenic protein.

A. Description of Alimentum

Alimentum is a nutritionally complete, hypoallergenic formula for infants (and a supplemental beverage for children) with severe food allergies, sensitivity to intact protein (including colic symptoms due to protein sensitivity), protein maldigestion or fat malabsorption. Alimentum is promoted specifically for those infants who are allergic to cow's milk protein and has been used successfully for the dietary management of CMPA in infants and toddlers since 1989. Alimentum is an exempt infant formula, which is used under the care of a physician or other health care professional. All labeling for Alimentum includes the statement "USE AS DIRECTED BY PHYSICIAN."

Alimentum is available in two forms: ready-to-feed (*i.e.*, liquid) and powder, with corresponding differences in overall ingredients. The ingredients in the ready-to-feed formulation are as follows: 87% water, 4.4% sugar (sucrose), 2.3% casein hydrolysate (enzymatically hydrolyzed), 2.2% modified tapioca starch, 1.4% safflower oil, 1.3% medium chain triglycerides, 1.1% soy oil; Less than 1% of: *C. cohnii* oil (a source of docosahexaenoic acid (DHA)), *M. alpina* oil (a source of arachidonic acid (ARA)), calcium citrate, calcium phosphate, carrageenan, potassium phosphate, ascorbic

acid, magnesium chloride, calcium hydroxide, potassium citrate, sodium chloride, L-cystine dihydrochloride, potassium chloride, L-tyrosine, choline chloride, L-tryptophan, ferrous sulfate, taurine, m-inositol, zinc sulfate, dl-alpha-tocopheryl acetate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, folic acid, potassium iodide, phylloquinone, biotin, sodium selenate, vitamin D_3 and cyanocobalamin.

The ingredients in the powder formulation of Alimentum are: 35.5% corn maltodextrin, 17.5% casein hydrolysate (enzymatically hydrolyzed), 14.5% sugar (sucrose), 9.7% high oleic safflower oil, 9.5% medium chain triglycerides, 8.0% soy oil; Less than 2% of: *C. cohnii* oil (a source of docosahexaenoic acid (DHA)), *M. alpina* oil (a source of arachidonic acid (ARA)), calcium phosphate, DATEM, potassium citrate, xanthan gum, magnesium chloride, mono- and diglycerides, sodium chloride, ascorbic acid, L-cystine dihydrochloride, calcium carbonate, L-tyrosine, potassium chloride, choline chloride, ferrous sulfate, L-tryptophan, taurine, m-inositol, ascorbyl palmitate, dl-alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, mixed tocopherols, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, potassium iodide, phylloquinone, biotin, sodium selenate, vitamin D_3 and cyanocobalamin.

B. Description of Extensively Hydrolyzed Casein

The primary protein source in both the ready to feed and powdered formulations of Alimentum – and the source of potential allergenic substances – is extensively hydrolyzed casein. The formula also is supplemented with free amino acids. The allergenic protein, casein, is hydrolyzed through the use of , which results in the breaking of the amino acid chains into free amino acids and smaller amino acid chains (polypeptides) that are unlikely to be allergenic. The ingredient specifications and manufacturing method/process flow for the extensively hydrolyzed casein used in Alimentum follows.

Ross Products Division Specifications For Extensively Hydrolyzed Casein Used In Alimentum

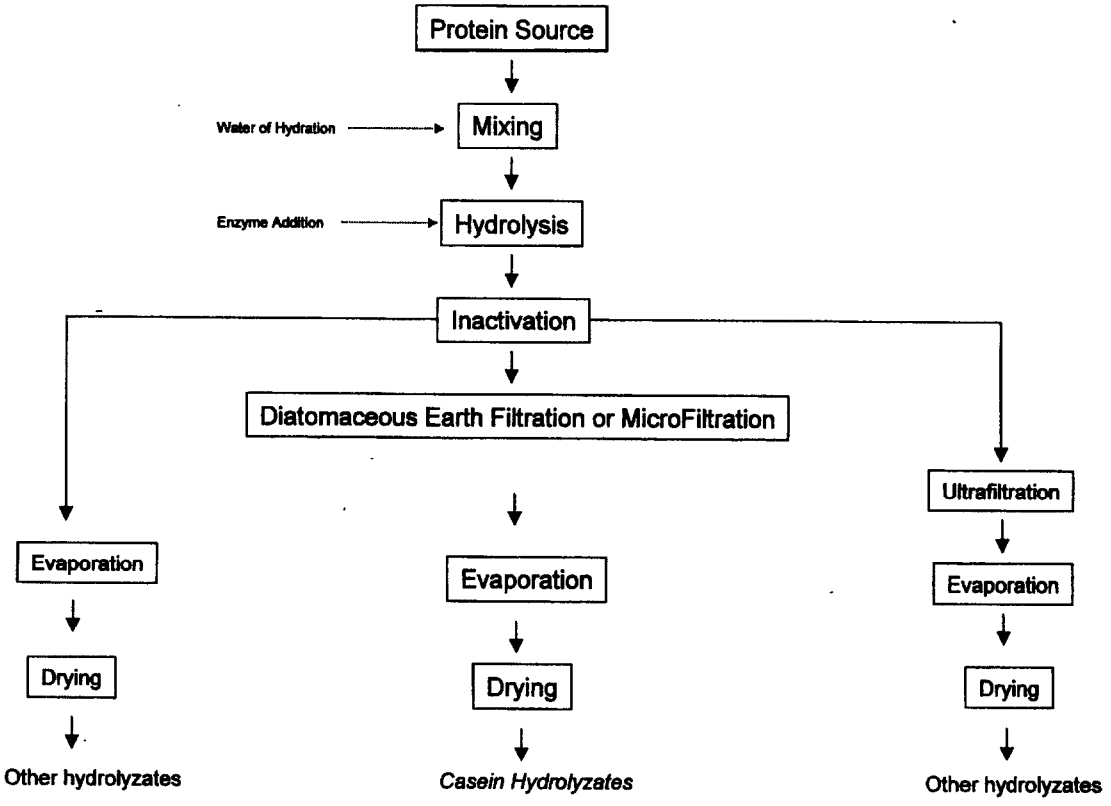
REDACTED

REDACTED

REDACTED

Manufacturing Flow For Extensively Hydrolyzed Casein Used In Alimentum

Manufacturing Process of Protein Hydrolyzates



REDACTED

C. Alimentum Manufacturing Process

In addition to the pervasive manufacturing controls that are in place during the manufacture of all infant formulas, Ross has implemented additional controls to address the unique issues presented by manufacturing a hypoallergenic infant formula. The controls begin with a thorough, validated and verified cleaning of the manufacturing equipment; strict quality assurance procedures for raw materials, with specific focus on the hypoallergenic status of the extensively hydrolyzed casein ingredient; as well as a variety of quality assurance procedures during and after the manufacture of Alimentum.

1. Preparation of Equipment Prior to the Manufacture of Alimentum

Prior to the manufacture of Alimentum, all manufacturing equipment is thoroughly cleaned using validated and documented cleaning procedures to assure that the manufacturing system is free from casein, whey, whole cow's milk, and soy protein. These procedures include multiple cycles of a caustic wash, a water rinse and an acid wash. Most equipment is cleaned using automated systems, but certain critical areas are cleaned by hand. All cleaning procedures have been validated and established as appropriate through Enzyme-Linked Immunosorbent Assay (ELISA) analyses of post-cleaning swab and rinse water samples for casein, whey and soy protein. Rinse water samples are obtained from sample ports or opened lines after cleaning procedures have been completed. Sampling is designed to represent all areas, and includes those considered more difficult to clean.

Re-verification of cleaning procedures is conducted whenever there have been significant changes to the equipment or system. Examples of significant changes include: new wet process equipment or systems, previously qualified equipment or systems that have been modified, or extension of the use of existing equipment/systems to the manufacture of Alimentum.

Additional steps are taken prior to manufacture of every batch of Alimentum powder. As part of the cleaning verification, swab samples of equipment, product contact surfaces and environmental areas are taken following completion of all cleaning procedures and prior to the manufacture of every batch of Alimentum. If results show that an area has not been sufficiently cleaned, it is re-cleaned, swabbed and tested until ELISA results indicate the area is clean.

The general protocol for the swab procedure is as follows. A representative surface area is swabbed, and the swab placed in a screw cap tube containing 5 mL of PBS with 0.1% Tween 20. If a piece of equipment is small, the entire surface area of that piece is swabbed. Sampling is designed to represent all areas, and includes areas considered more difficult to clean. The swab solution is tested for casein, whey and soy using validated ELISA methods. The validated ELISA methods used have

been previously published (Cordle *et al.*, 1994) and are currently in use with some minor modifications.11

Ross has implemented quality assurance procedures to ensure that Alimentum is hypoallergenic. Ross has collected information from the suppliers of each of the ingredients used in Alimentum to ensure that there are no milk-based ingredients in the raw materials. Ross also requires the suppliers of its raw materials to ensure that they have the appropriate controls in place to prevent cross contact with milk and other allergenic proteins.

The extensively hydrolyzed casein raw ingredient is tested by ELISA to assure that it meets or exceeds the standards necessary to produce a hypoallergenic finished product as established in the DBPCFC trial, described in the Clinical Testing section below. Furthermore, the manufacturing facilities perform sequential ELISA testing on Alimentum during processing to further assure hypoallergenic quality during the manufacturing process. The ELISA methods used to test the extensively hydrolyzed casein and the Alimentum formula are validated modifications of methods described in the literature (Cordle *et al.*, 1994; Cordle *et al.*, 1991). The modification implemented for finished product testing is that the casein, whey and soy standards and positive controls are diluted in a previously tested and qualified lot of Alimentum instead of in PBS with 0.1% Tween 20 and 0.05% ovalbumin. The use of Alimentum as diluent for the standards eliminates a difference in matrix between the standards and the sample, providing a standard curve with a slope that will measure the presence of casein, whey and soy in product samples with greater precision and accuracy.

The following additional quality assurance procedures are employed during the manufacture of Alimentum:

- Employees performing the work are trained to understand the importance of preserving a "protein-free" environment during manufacture of hypoallergenic products.

- Documented procedures are in place to limit employee access to "protein-free" areas.

- Mechanical systems are in place to support maintenance of a "protein-free" environment (*e.g.*, positive pressure, HEPA filtration of incoming air, "protein-free area" signage, etc.)

- The production line used to fill Alimentum powder is dedicated to "protein-free" products.

Following manufacture, ELISA testing is performed as part of the finished product release testing to assure that all commercial batches of Alimentum meet rigorous antigen content specifications to maintain its hypoallergenic clinical performance.

D. Scientific Data Demonstrating That Alimentum Does Not Contain Allergenic Protein

1. Background of "Hypoallergenic" Definition

In January 1990, the Nutrition and Allergic Disease Subcommittee of the AAP submitted a report to the FDA that established a definition of and requirements for hypoallergenic infant formulas (Subcommittee on Nutrition and Allergic Disease, 1990). Hypoallergenic infant formulas are intended for use by infants with existing CMPA symptoms. The AAP report was commissioned by FDA and the recommendations made therein have been published in the scientific literature (Committee on Nutrition, 1989; Committee on Nutrition, 2000; Kleinman, 1992). The report contained the following recommendations:

- Prior to conducting clinical trials with a test formula, comprehensive preclinical testing must demonstrate that the test formula is nutritionally suitable for infants and predict with reasonable certainty that the test formula will not cause reactions in milk-allergic infants and young children.

- The appropriate use of the "hypoallergenic" claim and safe use by infants and young children with CMPA must be established by clinical testing in the sensitive population. A DBPCFC trial followed by open challenge in an appropriate number of infants or young children with demonstrated CMPA must establish, with 95% statistical confidence, that at least 90% of milk-allergic infants will not react to the product.

Corresponding to the AAP recommendations, there are a number of preclinical and immunological test methods that are used to predict protein hydrolysate clinical performance in allergic infants (Cordle, 1994). In addition, DBPCFC described by the AAP, which is considered the "gold standard" in determining clinical hypoallergenic product performance in infants with CMPA, has been well documented for this purpose (Bock, 2000; Sampson, 1988; Bock & Atkins, 1990). Both the preclinical and clinical studies supporting the appropriate use of Alimentum in infants with CMPA have been published in the scientific literature. The results of these studies are described below.

2. *In Vitro* Testing

ELISA testing provides a quantitative measure of the antigenicity of hydrolyzed infant formulas and is recognized as a useful predictor of hypoallergenic performance (Isolauri *et al.*, 1995; Cordle, 1994). The use of ELISA allows for the determination of immunologically active antigen at concentrations as low as 10 ng/mL (Cordle, 1994).

ELISA testing has been used to document the level of immunologically active cow's milk protein in Alimentum (Sampson *et al.*, 1991; Businco *et al.*, 1993; Oldaeus *et al.*, 1991; Cordle *et al.*, 1994). The published results of the ELISA testing on Alimentum, Nutramigen®, another hypoallergenic formula containing extensively hydrolyzed casein, and Good Start®, a partially hydrolyzed whey protein formula not considered hypoallergenic, are displayed in Table 1. These results show that the residual antigenic protein (*i.e.*, whey, β-lactoglobulin, and casein) in Alimentum is substantially lower than that for the partially hydrolyzed formula and consistent with the other hypoallergenic formula.

Table – Published ELISA Results Documenting the Level of Immunologically Active Cow's Milk Protein in Alimentum, Nutramigen and Good Start

Publication	Formula	Whey	β-lactoglobulin	Casein	Units
Cordle *et.al.* 1994	Alimentum	20.7		7.68	mcg IAW or IAC/ g protein*
	Nutramigen[1]	25.6		12.2	
	Good Start[2]	214,000		1060	
Businco *et.al.* 1993	Alimentum		<0.01		ng/mL formula
	Nutramigen		0.006		
	Good Start		12,400		
Sampson *et.al.* 1991	Alimentum	1.27		0.19	mcg/mL formula
	Nutramigen	0.40		0.07	
	Good Start	751.9		24.2	
Oldaeus *et.al.* 1991	Alimentum		0.03		mcg/g dry weight
	Nutramigen		0.006		

*Abbreviations used: mcg, microgram; IAW, immunologically active whey; IAC, immunologically active casein; g, gram; ng, nanogram; mL, milliliter
[1]Manufactured by Mead Johnson Nutritionals.
[2]Manufactured by Nestle USA, Inc.

3. Animal Models of Hyperimmunization

a) Rabbit Model

A sensitive model of hyperimmunization was used to assess the relative immunoreactivity of the casein hydrolysate ingredient used in Alimentum (Cordle *et al.*, 1991). The purpose of the study was to establish a rigorous animal model that could be used to test the potential allergenic reactivity of extensively hydrolyzed casein in order to

select a protein hydrolysate ingredient that would provide hypoallergenic clinical performance. Hydrolyzed soy protein was also tested. New Zealand white rabbits were fed a casein-free diet for 14 days prior to immunization with one of two doses of the extensively hydrolyzed casein used in Alimentum (5 and 100 mg/rabbit/immunization) or immunization with 5 mg intact casein. Each rabbit received three immunizations: a primary immunization on day 1, and booster immunizations at days 22 and 43. ELISA was used to quantitate the antibody response in serum samples of each experimental group. Rabbits immunized with intact casein demonstrated anti-casein antibody titers consistent with expected immune response kinetics and reached a peak group mean antibody titer of 5.4×10^7 mg/rabbit at day 49. The response demonstrated by the rabbits immunized with the extensively hydrolyzed casein ingredient was extremely low, peaking at 52 mg/rabbit at day 28. There was no difference between the anti-casein hydrolysate titers of animals injected with 5 or 100 mg protein equivalent/rabbit. Cross-reactivity titers of hydrolysate and intact protein immunized animals were also very low throughout the study period. The results of this study demonstrate that the extensively hydrolyzed casein used to manufacture Alimentum exhibits extremely low immunogenicity relative to its parent protein, intact casein.

In a subsequent study, the model described above was expanded to test infant formulas (finished products show that hypoallergenic infant formulas based on extensively hydrolyzed casein generate weak immune responses relative to partially hydrolyzed whey-based infant formulas which are not hypoallergenic (Cordle *et al.*, 1994). Groups of rabbits were hyperimmunized with one of the following: Alimentum, Nutramigen®12 (hypoallergenic), Pregestimil® (hypoallergenic), Good Start®13 (not hypoallergenic), Similac with Iron and Enfamil with Iron (intact cow milk-based). This study demonstrated that rabbits immunized with known hypoallergenic infant formulas (*i.e.*, Alimentum, Nutramigen, and Pregestimil) generate very weak immune responses (<100 fold antibody increases) as compared to rabbits immunized with the partially hydrolyzed whey-based infant formula (>10,000 fold increase). These data were correlated with actual analytical values for residual immunologically active casein and whey in order to validate the use of the rabbit hyperimmunization model for predicting allergenic reactivity of formulas prior to conducting human studies.

b) Guinea Pig Model

Alimentum also has been examined using the guinea pig model of oral sensitization (Cordle, 2004). Although this model is not as sensitive as the rabbit hyperimmunization model, it is useful because it simulates oral sensitization to allergens. Guinea pigs were fed one of five different formulas, including Alimentum for 33 days. On day 35, the animals were administered a rapid systemic antigen challenge and observed for anaphylactic response. Animals fed Alimentum did not become sensitized to cow's milk protein as evidenced by the absence of allergic symptoms. All animals fed

12 Nutramigen and Pregestimil are manufactured by Mead Johnson Nutritionals.

13 Manufactured by Nestle USA, Inc.

intact cow's milk-based formulas exhibited strong immune responses resulting in fatal anaphylactic reactions. Animals fed a partially hydrolyzed whey-based formula exhibited weaker but still significant allergic responses.

4. Clinical Testing

As mentioned previously, the DBPCFC trial serves as the clinical standard for hypoallergenic determinations (Kleinman *et al.*, 1991). Alimentum has been clinically documented as hypoallergenic in a DBPCFC trial (Sampson *et al.*, 1991), and this study has been acknowledged by the AAP as an example of clinical testing for a product that has met the standard for hypoallergenicity (Committee on Nutrition, 2000).

In this study, twenty-five infants with documented immediate-type sensitivity to cow's milk protein were admitted to the Johns Hopkins Pediatric Clinical Research Unit for participation in the study. Subjects were fed either 10 grams of dehydrated cow's milk or powered Alimentum in 100 milliliters of Nutramigen®, which was used as a test substance carrier and a placebo-control. Subjects who had no reaction to the Alimentum test formula were openly fed up to 240 milliliters of the formula during a subsequent observation period. Following the observation period, subjects were given Alimentum to consume at home.

The challenge studies were conducted over a two day period. The subjects received one test food (Alimentum or dehydrated cow's milk) and one placebo (Nutramigen) on each of two days of observation in the clinical research unit. Three different commercial lots of Alimentum were used in the study.

Twenty-three of the 25 subjects reacted to the cow's milk protein. The reactions included cutaneous symptoms in 16 subjects, gastrointestinal symptoms in 11 subjects, and respiratory symptoms in 15 subjects. No significant food allergy symptoms were observed during the blinded Alimentum challenges, and all 25 subjects tolerated the supervised open feeding without problems.

This study served to document the hypoallergenicity of Alimentum. Furthermore, the level of the residual immunologically active cow's milk proteins contained in Alimentum used in the DBPCFC trial was documented and serves as a hypoallergenic standard by which the quality of commercial batches of Alimentum is assessed.

E. Analytical Method

FALCPA requires a description of the analytical method that is used to demonstrate that the ingredient does not contain allergenic protein. The hypoallergenicity of Alimentum is based on the ELISA analytical testing described above and confirmed through the DBPCFC trial demonstrating that Alimentum meets the AAP criteria for hypoallergenic infant formulas. The ELISA testing, when combined with the

clinical study, provide the method used to demonstrate that Alimentum does not contain "allergenic protein."

F. FDA Recognition of Rigor of Hypoallergenic Infant Formulas

The DBPCFC trial not only validates the hypoallergenicity of infant formula in accordance with the AAP testing protocol, but, as explained below, FDA has recognized the use of this protocol as an appropriate application of the scientifically rigorous, quantitative risk assessment-based approach for establishing a threshold for food allergens.

As part of FDA's on-going risk management of food allergens and in response to the FALCPA, the agency's Center for Food Safety and Applied Nutrition (CFSAN) established an internal, interdisciplinary group, the Threshold Working Group. FDA established this group "to evaluate the current state of scientific knowledge regarding food allergies and celiac disease, to consider various approaches for establishing thresholds for food allergens and for gluten, and to identify the biological concepts and data needed to evaluate the scientific soundness of each approach." In its June 2005 draft report, the Threshold Working Group identified four approaches that could be used to establish thresholds for allergens:

- *Analytical methods-based thresholds*, which are determined by the sensitivity of the analytical method(s) used to verify compliance;
- *Safety assessment-based thresholds*, in which a "safe" level is calculated using the No Observed Adverse Effect Level (NOAEL) from available human challenge studies and an appropriate uncertainty factor applied to account for knowledge gaps;
- *Risk assessment-based thresholds*, in which known or potential adverse health effects resulting from human exposure to a hazard are examined and the levels of risk associated with specific exposures and the degree of uncertainty inherent in the risk estimate are quantified; and
- *Statutorily-derived thresholds*, which are created by an exemption articulated in an applicable law and extrapolated to other potentially similar situations.

The Threshold Working Group concluded that:

> Of the four approaches described, the quantitative risk assessment-based approach is the most rigorous and provides the insight into the level of risk associated with specific exposures and the degree of uncertainty inherent in the risk estimate. An example of the use of a risk estimate and associated

> uncertainty is the current standard for hypoallergenic infant formulas, where there is 95% certainty that 90% of the sensitive population will not react (internal citation omitted). The risk assessment-based approach is preferred when a biological threshold cannot be justified scientifically.

(TWG Draft Report at 35).

As demonstrated by the results of preclinical and clinical studies specified in the AAP protocol, Alimentum meets the definition of a hypoallergenic infant formula. In the DBPCFC trial, it was shown, with 95% certainty, that 90% of infants and children with CMPA will not react to Alimentum. The establishment of such a risk assessment-based threshold for milk-derived protein in infant formula is readily acknowledged by CFSAN's own internal working group as appropriate. Therefore, exemption of Alimentum from the FALCPA allergen labeling requirements is justified.

III. REFERENCE LIST

Bock, S. A. (2000). Evaluation of IgE-Mediated Food Hypersensitivities. *J Pediatr Gastroenterol Nutr* **30**, S20-S27.

Bock, S. A. & Atkins, F. M. (1990). Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. *J Pediatr* **117**, 561-567.

Businco, L., Dreborg, S., Einarsson, R., Giampietro, P. G., Høst, A., Keller, K. M., Strobel, S., & Wahn, U. (1993). Hydrolysed cow's milk formulae. Allergenicity and use in treatment and prevention. An ESPACI position paper. *Pediatr Allergy Immunol* **4**, 101-111.

Committee on Nutrition, American Academy of Pediatrics (1989) Hypoallergenic infant formulas. *Pediatrics* **83**, 1068-1069.

Committee on Nutrition, American Academy of Pediatrics (2000). Hypoallergenic infant formulas. *Pediatrics* **106**, 346-349.

Cordle, C. T. (1994). Control of food allergies using protein hydrolysates. *Food Tech* **48**, 72-76.

Cordle, C. T. (2004). Soy protein allergy: incidence and relative severity. *J Nutr* **134**, 1213S-1219S.

Cordle, C. T., Duska-McEwen, G., Janas, L. M., Malone, W. T., & Hirsch, M. A. (1994). Evaluation of the immunogenicity of protein hydrolysate formulas using laboratory animal hyperimmunization. *Pediatr Allergy Immunol* **5**, 14-19.

Cordle, C. T., Mahmoud, M. I., & Moore, V. (1991). Immunogenicity evaluation of protein hydrolysates for hypoallergenic infant formulae. *J Pediatr Gastroenterol Nutr* **13**, 270-276.

Isolauri, E., Sütas, Y., Mäkinen-Kiljunen, S., Oja, S. S., Isosomppi, R., & Turjanmaa, K. (1995). Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. *J Pediatr* **127**, 550-557.

Kleinman, R. E. (1992). Cow milk allergy in infancy and hypoallergenic formulas. *J Pediatr* **121**, S116-S121.

Kleinman, R. E., Bahna, S., Powell, G. F., & Sampson, H. A. (1991). Use of infant formulas in infants with cow milk allergy: A review and recommendations. *Pediatr Allergy Immunol* **4**, 146-155.

REDACTED

Oldæus, G., Björkstén, B., Einarsson, R., & Kjellman, N. I-M. (1991). Antigenicity and allergenicity of cow milk hydrolysates intended for infant feeding. *Pediatr Allergy Immunol* **4**, 156-164.

Sampson, H. A. (1988). Immunologically mediated food allergy: the importance of food challenge procedures. *Annals of Allergy* **60**, 262-269.

Sampson, H. A., Bernhisel-Broadbent, J., Yang, E., & Scanlon, S. M. (1991). Safety of casein hydrolysate formula in children with cow milk allergy. *J Pediatr* **118**, 520-525.

Subcommittee on Nutrition and Allergic Disease. (1990) Infant Formulas and Allergic Disease. American Academy of Pediatrics. FDA contract 223-86-2117.

REDACTED